

# Correlation of IHC and FISH for *ALK* Gene Rearrangement in Non-small Cell Lung Carcinoma

## IHC Score Algorithm for FISH

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**Introduction:** Accurate, cost-effective methods for testing anaplastic lymphoma kinase gene rearrangement (*ALK*+) are needed to select patients with non-small cell lung carcinoma for *ALK*-inhibitor therapy. Fluorescent in situ hybridization (FISH) is used to detect *ALK*+, but it is expensive and not routinely available. We explored the potential of an immunohistochemistry (IHC) scoring system as an affordable, accessible approach.

**Methods:** One hundred one samples were obtained from an enriched cohort of never-smokers with adenocarcinoma from the Mayo Clinic Lung Cancer Cohort. IHC was performed using the

*ALK*1 monoclonal antibody with ADVANCE detection system (Dako) and FISH with dual-color, break-apart probe (Abbott Molecular) on formalin-fixed, paraffin-embedded tissue.

**Results:** Cases were assessed as IHC score 0 (no staining;  $n = 69$ ), 1+ (faint cytoplasmic staining,  $n = 21$ ), 2+ (moderate, smooth cytoplasmic staining;  $n = 3$ ), or 3+ (intense, granular cytoplasmic staining in  $\geq 10\%$  of tumor cells;  $n = 8$ ). All IHC 3+ cases were FISH+, whereas 1 of 3 IHC 2+ and 1 of 21 IHC 1+ cases were FISH+. All 69 IHC 0 cases were FISH-. Considering FISH a gold-standard reference in this study, sensitivity and specificity of IHC were 90 and 97.8%, respectively, when 2+ and 3+ were regarded as IHC positive and 0 and 1+ as IHC negative.

**Conclusions:** IHC scoring correlates with FISH and may be a useful algorithm in testing *ALK* by FISH in non-small cell lung carcinoma, similar to human epidermal growth factor-2 testing in breast cancer. Further study is needed to validate this approach.

**Key Words:** Immunohistochemistry, Fluorescent in situ hybridization, Anaplastic lymphoma kinase, Non-small cell lung carcinoma, Adenocarcinoma.

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blocked by small-molecule inhibitors that target ALK, which supports a role for EML4–ALK as a key driver of lung tumorigenesis in a subset of NSCLCs<sup>7,9</sup>; thus, this fusion oncogene represents one of the newest molecular targets in NSCLC.

In a phase I clinical trial, investigational drug crizotinib (PF-02341066), an ALK inhibitor, showed an objective response rate of 57% and disease control rate of 87% at 8 weeks in patients with NSCLC whose tumors had a rearrangement of *ALK*.<sup>10</sup> Therefore, it is critical to identify patients with this abnormality correctly. A true gold standard for *ALK*<sup>+</sup> has not been established, although fluorescent in situ hybridization (FISH) has been used to detect *ALK*<sup>+</sup> tumors in clinical trials and in a previous study of clinical features and outcomes of patients with *ALK*<sup>+</sup> NSCLC.<sup>11</sup> However, FISH is relatively expensive and not widely available. Immunohistochemistry (IHC) is relatively inexpensive, faster, and performed routinely in most diagnostic laboratories; thus, it could serve as a practical tool for clinicians.

We hypothesized that IHC can be used to screen for *ALK*<sup>+</sup> with confirmatory FISH based on IHC scoring, a system well established in human epidermal growth factor receptor-2 (HER2) testing for patients with breast cancer.<sup>12</sup> We report an IHC scoring system based on a routine IHC method with a widely used, commercially available antibody for ALK. Such a practical screening method, in combination with a confirmatory test—in this case, FISH—might significantly reduce the need to perform FISH to detect *ALK*<sup>+</sup> status in patients with NSCLC, thereby substantially decreasing costs and turnaround times.

## MATERIALS AND METHODS

### Case Selection

The Mayo Clinic Lung Cancer Cohort was used to identify patients for this retrospective study. Given the low incidence of *ALK*<sup>+</sup> reported in resected lung adenocarcinomas within a nonselected western population<sup>13</sup> and that previous studies have reported that adenocarcinomas harboring *ALK*<sup>+</sup> tend to occur in never-smokers or light smokers,<sup>11,13,14</sup> we enriched our cohort by including only never-smokers with adenocarcinoma. A total of 101 patients met the selection criteria and were included in this study.

Patients with adenocarcinoma who had never smoked, and had a minimum of 1 year of follow-up medical records, and banked tissue samples from surgical resections (composed of wedge resection or more extensive surgeries), were eligible for inclusion. Whole sections, not tissue microarrays, were used in this study. All available slides from each case were reviewed to verify the diagnosis and to select the representative block containing most viable tumor cells by two pathologists (E.S.Y. and M.C.A.). This study has been approved by the institutional review board of the Mayo Foundation.

### Immunohistochemistry

Four-micron sections cut from formalin-fixed, paraffin-embedded blocks were placed on charged slides, which were then dried and melted in a 62°C oven for 20 minutes. Slides

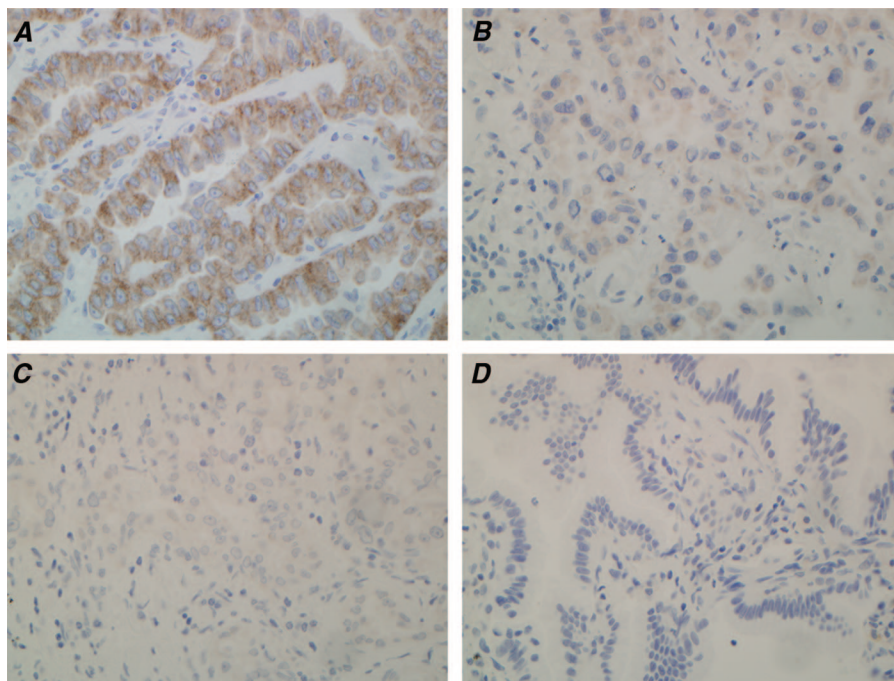
were deparaffinized through xylenes and graded alcohols to tap water, and heat-induced epitope retrieval was performed by treating the slides in a Dako PT Link (Dako, Carpinteria, CA), containing a solution of 1 mM ethylenediaminetetraacetic acid, pH 8.0, preheated to 97°C, for 30 minutes. All remaining steps were performed at room temperature. Endogenous peroxidase was blocked by placing the slides in a 1:1 solution of 3% hydrogen peroxide: absolute methanol. Slides were placed on the Dako Autostainer (Dako); primary antibody (mouse monoclonal ALK antibody, clone ALK1; Dako; 1:100 dilution) was applied and incubated for 30 minutes. Antigen–antibody reaction was visualized by an enhanced polymer-based detection system, ADVANCE (Dako), with 20-minute incubation for ADVANCE Link and 20-minute incubation for ADVANCE horseradish peroxidase. Diaminobenzidine (DAB<sup>+</sup>; Dako) was employed for 5 minutes as the chromogen. All slides were counterstained with hematoxylin, dehydrated, and coverslipped for microscopic examination. The positive control was from a known CD30-positive ALCL case. The negative control was a mouse immunoglobulin G1 serum substitution for the primary antibody (ALK).

### IHC Scoring

An IHC score was assigned to each case according to the following criteria: 3+, intense, granular cytoplasmic staining; 2+, moderate, smooth cytoplasmic staining; 1+, faint cytoplasmic staining in ≥10% of tumor cells; and 0, no staining. IHC scoring was performed by two pathologists (E.S.Y. and J.M.B.) before FISH testing. IHC score 3 cases typically reveal widespread, although still patchy, easily discernable intense cytoplasmic positivity that shows a distinct granular texture as depicted in Figure 1A. IHC score 2 cases also show readily recognizable positivity but to a lesser extent and with less intensity of staining than in score 3 cases. Score 2 cases do not reveal the granular texture seen in score 3 cases but show more smooth or smudged staining (Figure 1B). In score 1 cases, the observer typically struggles to determine whether faint positivity represents a background stain or real positivity, unlike the readily detectable staining of score 2 and 3 cases (Figure 1C). Score 0 cases do not show any significant staining (Figure 1D). Consensus was established by reviewing the slides together in the case of discrepant scores, typically 1+ versus 0. To evaluate the reproducibility of our scoring system, two additional pathologists (A.C.R. and J.J.M.) performed IHC scoring after a training session, with typical examples of each score provided by the pathologist who devised the scoring scheme (E.S.Y.). E.S.Y. repeated the scoring along with the two additional pathologists for intraobserver and interobserver reproducibility. The kappa statistic was used for interobserver variability between the findings of all three pathologists (E.S.Y., A.C.R., and J.J.M.) and intraobserver variability within the findings of a single pathologist (E.S.Y.).

### FISH for *ALK* Rearrangement

Interphase molecular cytogenetic studies using a commercially available *ALK* probe (Vysis LSI *ALK* Dual Color, Break Apart Rearrangement Probe; Abbott Molecular, Abbott Park, IL) were performed on 4-μm paraffin-embedded

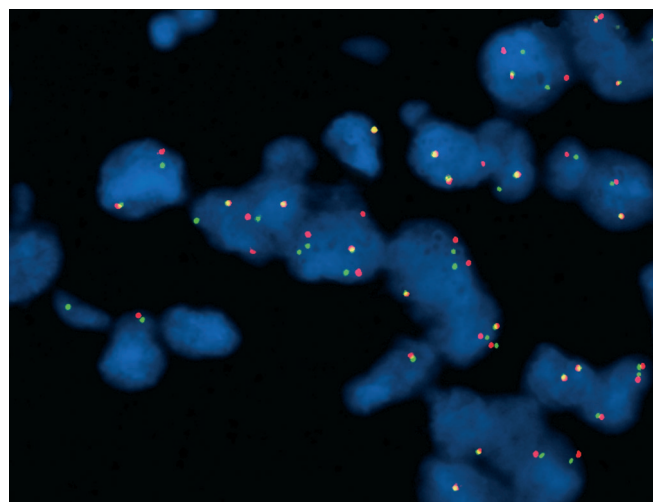


**FIGURE 1.** Score 3 showing intense, granular cytoplasmic staining (A); score 2 showing moderate, smooth (without the apparent granularity seen in score 3) cytoplasmic staining (B); score 1 showing faint, barely discernable cytoplasmic staining (C); and score 0 showing no staining (D). Anaplastic lymphoma kinase immunohistochemical staining,  $\times 200$  original magnification.

sections that were deparaffinized twice in xylene for 15 minutes, dehydrated twice in 100% ethylic alcohol for 5 minutes, and treated with 10 mmol/L citric acid for 10 minutes in a humid microwave. Tissue sections were then transferred to 37°C 2 $\times$  standard saline citrate (SSC) for 5 minutes, and protein was digested with Digest All-3 (Zymed, San Francisco, CA). After brief washing in 1 $\times$  phosphate-buffered saline, the slides were sequentially dehydrated in alcohol (70, 85, and 100%) and air dried at room temperature. Five microliters of ALK probe were diluted per manufacturer's instructions and added to the tissue sections. Slides were denatured at 80°C for 5 minutes, and probe hybridization was carried out overnight in a humidified chamber at 37°C. Tissue sections were washed in 0.1% NP40/2 $\times$  SSC at 76°C for 4 minutes and then washed in 0.1% NP40/2 $\times$  SSC at room temperature for 1 minute. Slides were mounted in VECTASHIELD Mounting Medium (Vector Laboratories, Burlingame, CA) with 1.5  $\mu$ g/ml of 4',6-diamidino-2-phenylindole.

### FISH Interpretation

FISH for *ALK* locus rearrangement was considered positive if 15% or more tumor cells counted showed a split signal of the fluorescent probes flanking the *ALK* locus (Figure 2). One hundred cells were analyzed in each case. All FISH interpretation was done in a separate laboratory without the knowledge of IHC results for ALK. Because the rearrangement of the *ALK* locus in this group of tumors is often because of a paracentric inversion of approximately 12.4 Mb (fusion with the *EML4* gene on chromosome 2p21), internal and external normal controls were used to estimate whether the distance between the separated signals was larger than the distance that would be expected for stochastic separation of these signals in tissues that lack *ALK* rearrangement. This



**FIGURE 2.** Dual-color, break-apart fluorescent in situ hybridization was performed on paraffin-embedded tissues using the commercially available anaplastic lymphoma kinase (ALK) probe (Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe; Abbott Molecular). The centromeric (green) and telomeric (red) flank the *ALK* locus. Splitting of the red and green signals indicates ALK rearrangement.

distance was estimated using the 1 signal size diameter as a reference. On the basis of a series of control experiments, we confirmed that probe separation distances larger than 8 Mb can be readily detectable on paraffin-embedded tissues with high sensitivity (95.6%) and specificity (97.6%).

The ALK probe consists of one red and one green signals that appear as yellow when fused in normal cells as opposed to two separate signals in abnormal cells. All patterns of red and green signals encountered in each of the 100



cells counted were recorded. We considered several different patterns of split signals as abnormal, and the number of abnormal cells was summed to generate the final count for determining FISH status. A threshold was set for each abnormal pattern at 5 or 10, and the number of abnormal cells was added if the number was above the threshold. In addition to the classic break-apart pattern (BAP) showing one fusion, one red, and one green signal in a cell, we also considered the following patterns as abnormal split signals: one fusion + one red signal only, one fusion + one green signal only, one to two fusion signals + two to three green signals + two to three red signals, one red + one green signal only (without fusion signal), one to two fusions + one to four red signals, two fusions + one BAP, three fusions + one BAP, and two to four fusions + one to two red signals. We set the threshold for patterns to be considered abnormal at 10% for classic BAP pattern and for one fusion + one red or green signal, and at 5% for the rest of patterns listed earlier. Then, we summed the numbers that were above the threshold, and this was regarded as FISH positive if it was 15 or higher. Representative data on positive and negative cases for FISH are listed in Supplementary Table 1 (Supplemental Digital Content 1, <http://links.lww.com/JTO/A63>).

## RESULTS

We found 10 (9.9%) *ALK*+ cases by FISH in our enriched cohort of never-smokers with adenocarcinoma (higher than reported in resected lung adenocarcinomas in an unselected cohort of western patients [1 of 227 cases; 0.45%]<sup>13</sup>). The mean age of the 10 patients *ALK*+ by FISH was 56 years (range 36–76 years). Five of the 10 patients were men. Three patients had stage I disease, one stage II, five stage III, and one stage IV. A more detailed clinicopathologic study, including examination of clinical outcomes in *ALK*+ versus carefully matched *ALK*– patients, is in progress with additional patients from the ongoing Mayo Clinic Lung Cancer Cohort, results of which will be reported separately.

IHC score 3+ was found in 8 cases, 2+ in 3 cases, 1+ in 21 cases, and 0 in 69 cases. All 8 IHC 3+ cases were *ALK*+ by FISH, whereas 1 of 3 IHC 2+ and 1 of 21 IHC 1+ cases were *ALK*+ by FISH. All 69 IHC 0 cases were negative for *ALK* by FISH. These findings are summarized in Table 1.

IHC 3+ and 2+ cases demonstrated readily appreciable cytoplasmic positivity (Figures 1A, B) and were thus regarded IHC positive. Conversely, IHC 1+ cases showed only faint positivity (Figure 1C) and were thus regarded IHC negative, as were IHC 0 cases without any discernable staining (Figure 1D). With this definition, there were two IHC-positive cases (both IHC 2+) that were negative for *ALK*

rearrangement by FISH, resulting in 97.8% specificity of IHC positivity for *ALK*+. However, all IHC 3+ cases were *ALK*+. One of 21 IHC 1+ cases was *ALK*+ by FISH, giving 90% sensitivity of IHC for *ALK*+. FISH on all 69 IHC 0 cases was also negative for *ALK* rearrangement.

The weighted kappa scores between E.S.Y. (original scorer) and A.C.R. or J.J.M. were 0.55 (95% confidence interval [CI] 0.40–0.70) and 0.50 (95% CI 0.35–0.64), respectively. Intraclass correlation in these three observers was 0.93 (95% CI 0.86–0.93). The weighted kappa between A.C.R. and J.J.M. was 0.80 (95% CI 0.72–0.89). The weighted kappa between the initial scores and the repeat scores by the reevaluation of E.S.Y. was 0.82 (95% CI 0.72–0.93). IHC scores by all observers on all cases along with FISH results are listed in Supplemental Table 2 (Supplemental Digital Content 2, <http://links.lww.com/JTO/A64>).

## DISCUSSION

We performed this study to evaluate the potential role of IHC as a detection or screening method for *ALK*+. We refined the IHC results by giving a score in each case and correlated this with *ALK*+ status by FISH in all cases. There was virtually no background staining in our IHC, consistent with the biologic fact that there is no constitutive *ALK* expression in the nonneoplastic lung tissue.<sup>7</sup>

A true gold standard to determine *ALK*+ has not been established. Various molecular techniques including reverse-transcriptase polymerase chain reaction, direct sequencing, and FISH have been used in previous studies (Table 2). FISH could be a method of choice, because it is relatively simpler than other molecular techniques and has the ability of directly visualizing translocation with appropriate probes for the *ALK* gene. However, FISH has its own pitfalls; interpretation of FISH for *ALK*+ in NSCLC tends to be more difficult than in ALCL or IMT because *ALK*+ in NSCLC is an intrachromosomal rearrangement, resulting in a relatively close separation of the break-apart probes, as shown in Figure 2. Rodig et al.<sup>13</sup> have also pointed out that FISH alone as initial screening did not detect all cases with *ALK*+. Previous studies<sup>11,13,16</sup> used a cutoff value of 15% or more tumor cells to be abnormal for FISH positivity. We also used 15% as the cutoff for FISH positivity among the 100 cells counted. However, none of the previous studies specified the number of tumor cells that they counted.<sup>11,13,16</sup> Most previous studies did not systematically perform IHC with other molecular techniques. Rather, they either performed IHC only on the *ALK*+ cases by a molecular technique or did molecular confirmation only on the IHC-positive cases, which precludes the assessment of sensitivity and specificity. In a previous study,<sup>15</sup> we used the same IHC technique as in this study but interpreted results only as positive or negative without scoring. Among 335 nonselected cases of NSCLC, there were 6 IHC-positive cases (IHC score 2+ or 3+ according to our current scoring criteria), all of which were positive for *ALK*+ by FISH. The specificity and sensitivity of IHC positivity for *ALK*+ could not be determined, because we did not perform FISH in all IHC-negative cases (IHC score 0 and 1+ according to our current scoring criteria). However, eight randomly selected

**TABLE 1.** Correlation of IHC and FISH

	IHC Score 3	IHC Score 2	IHC Score 1	IHC Score 0
FISH+ ( <i>n</i> = 10)	8	1	1	0
FISH– ( <i>n</i> = 91)	0	2	20	69

IHC, immunohistochemistry; FISH, fluorescent in situ hybridization.

**TABLE 2.** ALK IHC Methods and Results in the Literature

Author	Tissue Type/ Section	Antibody Source	Clone	Dilution	Pretreatment/ Detection Method	Gold Standard for ALK+	Sensitivity	Specificity
This study	Paraffin/whole section	Dako	ALK1	1:100	HIER with EDTA (pH 8.0), in Dako PT link; ADVANCE (Dako)	FISH	90.0%	97.8%
Boland et al. <sup>15</sup>	Paraffin/whole section	Dako	ALK1	1:100	HIER with EDTA (pH 8.0), in Dako PT link; ADVANCE (Dako)	FISH, RT-PCR, gene expression profiling	100% <sup>a</sup> (tested in 40 of 335 cases)	100%
Rodig et al. <sup>13</sup>	Paraffin/TMA	Dako	ALK1	1:2	HIER with EDTA (pH 8.0), in pressure cooker (Decloaking Chamber [Biocare Medical]); tyramide amplification and EnVision+ (Dako)	FISH	80%	100%
Mino-Kenudson et al. <sup>16</sup>	Paraffin/mostly TMA (116 of 153 cases)	Dako for ALK1, Cell Signaling Technology (Danvers, MA) for D5F3	ALK1, D5F3	1:2 for ALK1, 1:100 for D5F3	HIER with EDTA (pH 8.0) in pressure cooker (Decloaking Chamber [Biocare Medical]), EnVision+ (Dako)	FISH	67% with ALK1 antibody, 100% with D5F3 antibody	97% with ALK1 antibody, 99% with D5F3 antibody
Wong et al. <sup>17</sup>	Paraffin/not specified	Invitrogen (Carlsbad, CA)	Polyclonal	1:1000	HIER with citrate (pH 6.0) in microwave; Dako HRP complex, not further specified	RT-PCR, direct sequencing	100% (tested in 12 of 13 ALK+ cases by molecular methods)	N/A
Shaw et al. <sup>11</sup>	Paraffin/not specified	Dako	ALK1	N/A	N/A	FISH	100%	N/A
Inamura et al. <sup>14</sup>	Paraffin/not specified	Dako	ALK1	1:20	HIER with Target Retrieval Solution (pH 9.0, Dako), EnVision + DAB system with modification <sup>b</sup>	Multiplex RT-PCR	100%	N/A
Takeuchi et al. <sup>18</sup>	Paraffin/not specified	Abcam (Cambridge, UK) for 5A4, Dako for ALK1	5A4 and ALK1	1:50	HIER with Target Retrieval system (pH 9.0, Dako); EnVision + DAB and iAEP method	Inverse and multiplex RT-PCR	100% for both antibodies	100% for both antibodies
Martelli et al. <sup>19</sup>	Paraffin/not specified	Not specified for ALK1 and ALKc, Thermo Fisher Scientific (Fremont, CA) for 5A4	ALK1, ALKc, 5A4	Not specified	HIER with citrate (pH 6.0) or EDTA (pH 8.0) in microwave; Dako-REAL, alkaline-phosphatase/ RED detection system	RT-PCR, FISH	0% tested	0% tested (nontumor tissue in the section stained)

<sup>a</sup> It was assumed that all ALK+ cases by FISH were positive for IHC (i.e., 100% sensitivity) based on the statement in the abstract that reads “*EML4-ALK* was identified by using FISH for *ALK* rearrangements and was confirmed by immunohistochemistry.” It was not further elaborated in the Results section, however.

<sup>b</sup> In the Materials and Method section, authors stated that “Immune complexes were detected with the EnVision+ DAB system (Dako) with minor modifications” and cited the study in press by Takeuchi et al. using iAEP method. Therefore, this study might have used the iAEP detection method as well.

HIER, heat-induced epitope retrieval; TMA, tissue microarray; HRP, horseradish peroxidase; iAEP, intercalated antibody-enhanced polymer; ALK, anaplastic lymphoma kinase; IHC, immunohistochemistry; FISH, fluorescent in situ hybridization; EDTA, ethylenediaminetetraacetic acid; RT-PCR, reverse-transcriptase polymerase chain reaction.

IHC-negative tumors were negative for *ALK* rearrangement by FISH in that study. Furthermore, 35 adenocarcinoma cases among the total 335 NSCLCs had available gene expression profile data, and IHC results were 100%

concordant with the gene expression profile. Two cases with transcriptional up-regulation of *ALK* were positive for *ALK* by IHC, whereas the remaining 33 cases were negative for *ALK* by IHC.<sup>15</sup>

Previous studies have reported a difficulty in detecting ALK fusion proteins by IHC, possibly due to a weak transcriptional activity of the promoter-enhancer region of *EML4* that drives the expression of EML4–ALK compared with that of the nucleophosmin promoter involved in the expression of the nucleophosmin–ALK fusion protein in ALCL.<sup>16,18</sup> Our protocol at Mayo and protocol of another group<sup>18</sup> showed a high sensitivity of ALK IHC using the polymer-enhanced detection system. Mino-Kenudson et al.<sup>16</sup> reported IHC with a novel, highly sensitive antibody for detection of ALK+ lung adenocarcinoma cases. However, this antibody is not commercially available and has not been independently tested by other researchers because of its limited availability. Mino-Kenudson et al.<sup>16</sup> applied different dilutions in ALCL and lung adenocarcinoma cases, which would not be practical in diagnostic laboratories; it will be cumbersome for technologists to apply different dilutions, requiring separate preparation of reagents, resulting in extra work and potential errors. Also, it could be problematic to use different clones of an antibody depending on the type of tumor; again, the extra step of indicating the clone of an antibody in each case could be a potential area of confusion or a cause of delay when omitted at the time of initial request.

In our study, we used a monoclonal antibody (Dako clone ALK1), one of the most commonly used, well-tested ALK antibodies, at the same dilution as used in ALCL and IMT, making its use more practical from a diagnostic laboratory perspective. Our IHC method is based on a standard IHC method for a routine diagnostic surgical pathology practice at Mayo Clinic Rochester for other antibodies and for the ALK antibody. Application of an enhanced polymer-based detection system (ADVANCE) may be the only deviation from standard laboratory methods that we have introduced. It has three steps, one more than conventional two-step systems such as EnVision+ (also marketed by Dako): (1) primary antibody application, (2) ADVANCE Link (goat antimouse/rabbit IgG), and (3) ADVANCE horseradish peroxidase, the enzyme-conjugated polymer backbone. According to the manufacturer, ADVANCE is at least five times more sensitive than EnVision+, which uses only two steps: primary antibody application and subsequent enzyme-conjugated secondary antibody application.

Takeuchi et al.<sup>18</sup> also reported that their intercalated antibody-enhanced polymer method, a method similar to the ADVANCE system we used, provided high sensitivity and specificity for ALK+ status proven by a polymerase chain reaction-based molecular technique (Table 2).

In breast cancer, IHC scoring for HER2 with the algorithm for FISH has been widely used in selecting patients for anti-HER2 therapy, with guidelines from the American Society of Clinical Oncology/College of American Pathologists.<sup>12</sup> According to the current American Society of Clinical Oncology/College of American Pathologists guidelines,<sup>12</sup> IHC 2+ is recommended to be confirmed by reflex FISH for *HER2* gene amplification, whereas HER2 IHC 0 or 1+ can be regarded as negative and 3+ as positive without FISH. Similarly, our IHC scoring system might be useful in evaluating patients with NSCLC for ALK-inhibitor treatment, which would significantly limit the need for FISH confirma-

tion and could reduce time and costs. The same algorithm could possibly be used (i.e., reflex FISH for IHC 2+ cases only), given the consistency of ALK+ in IHC 3+ and the low frequency of ALK+ in IHC 1+ and 0 cases (1 of 21 and none of 69, respectively). An alternative algorithm can be considered to direct IHC 1+ and IHC 2+ cases for reflex FISH. However, distinction of IHC 1+ from IHC 0 may be subjective, which could pose a problem for an algorithm requiring reflex FISH on IHC 1+ cases; many negative cases might be directed for FISH, which would decrease the time and cost benefit. In the context of HER2 testing in breast cancer, some IHC 1+ and 0 cases have been found to be FISH positive (seven of 100 and 3 of 100, respectively).<sup>6</sup> Therefore, we feel that the sensitivity of our IHC scoring would be acceptable to use the former algorithm (i.e., reflex FISH for IHC 2+ cases only). Also, discretionary FISH testing can be performed even on IHC 1+ or 0 cases if a clinical suspicion for ALK+ is high (e.g., younger age, nonsmoking status, signet-ring cell features). Currently, we are testing more cases in an ongoing clinicopathologic study drawing from the Mayo Lung Cancer Cohort; ALK testing by IHC and FISH will be further evaluated in these cases.

Our IHC scoring might be an inexpensive and accurate screening method, by an algorithm for FISH confirmation, to identify patients with ALK+ NSCLC who will benefit from anticipated anti-ALK therapy. However, further study is needed to confirm our results. Our IHC method used a time-tested, reliable monoclonal ALK antibody and a sensitive, automated detection system, both of which are commercially available.

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